## ORIGINAL ARTICLE

# A new species of Aequales, genus *Gieysztoria* (Rhabdocoela: Dalyelliidae) from China

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**Abstract** A new species of Aequales group, namely *Gieysztoria shiyanensis* Wang & Xia, **sp. nov.**, is described from China. The new species is diagnosed by a sclerotic stylet with a belt attaching 15 fang-like spines. A worldwide list of Aequales group and a key to species of *Gieysztoria* in China are provided.

**Key words** Turbellarian, Rhabdocoela, *Gieysztoria*, new species, China.

#### 1 Introduction

The genus *Gieysztoria* Ruebush & Hayes, 1939, which belongs to the Dalyelliidae Graff, 1908, is mostly limnic although a few species inhabit marine and brackish waters (Van Steenkiste *et al.*, 2012). *Gieysztoria* is a genus of miniature turbellarians of only 1–2 mm in length with an anterior end, a tapering posterior end, and a pair of eyes. The cask-like pharynx locate in the posterior of the cerebroganglion. *Gieysztoria* exhibits hermaphrodite and cross fertilization characteristics. Its sclerotic male organ comprises of a belt that bears numerous spines. This turbellarian inhabits non-poisonous freshwater wetlands and mainly preys on native animals and benthic rotifers. Approximately 90 species of *Gieysztoria* are known. Based mainly on the configuration and shape of the male style, the genus *Gieysztoria* can be classified into two groups—Aequales and Inaequales (Tyler *et al.*, 2012; Van Steenkiste *et al.*, 2012; Lai *et al.*, 2013; Lu *et al.*, 2013).

The genus *Gieysztoria* was first described in Southern Iceland, Europe in 1861, and was subsequently recorded in Europe, Southern America, and Australia, among others. The first description of *Gieysztoria* species in China was by An-Tai Wang in Shenzhen (Wang & Wu, 2005). To date, six *Gieysztoria* species have been described in China: *G. shenzhensis* Wang & Wu, 2005, *G. pulchra* Wang & Deng, 2006, *G. macrovariata* 9-spinosa Luther, 1955, *G. wuyishanensis* Wang & Lai, 2013, *G. bimaculata* Wang, Lu & Wu, *G. guangdongensis* Wang & Xia.

In this report, we describe one new species of the Aequales group collected from a wetland in Shiyan Reservoir in Shenzhen, China.

#### 2 Materials and methods

The specimens were saved in Bouin's solution and stained by haematoxylin-eosin (HE) method. Some were whole-mounted with polyvinyl-lactophenol for dissection. The flatworms were observed in vivo with help of a

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stereomicroscope (Leica MZ16). Photographs were captured using OLYMPUS BX51 and BX53 microscope equipped with an OLYMPUS DP72 camera. Samples were measured with Image Pro Plus software version 6.0 (Media Cybernetics Inc.). Figures and photographs were post-corrected with Adobe Photoshop® version 7.0. Illustrations were drawn according to the digital photographs.

#### 3 Results

#### Gieysztoria shiyanensis Wang & Xia, sp. nov. (Figs 1–9)

Material examined. Holotype PLA-G00071, wetlands of Shiyan Reservoir, Shenzhen, Guangdong, China (22°40′N, 113°53′E), 19 June 2013, coll. Yan-Hong Lu, saved in Bouin's solution and stained by HE. Paratypes PLA-G0072–PLA-G0075, same data as holotype. All materialswere deposited at Institute of Zoology, Chinese Academy of Sciences (IZCAS), Beijing, China.

Etymology. The specific name refers to the type locality of the species.

### 3.1 Description

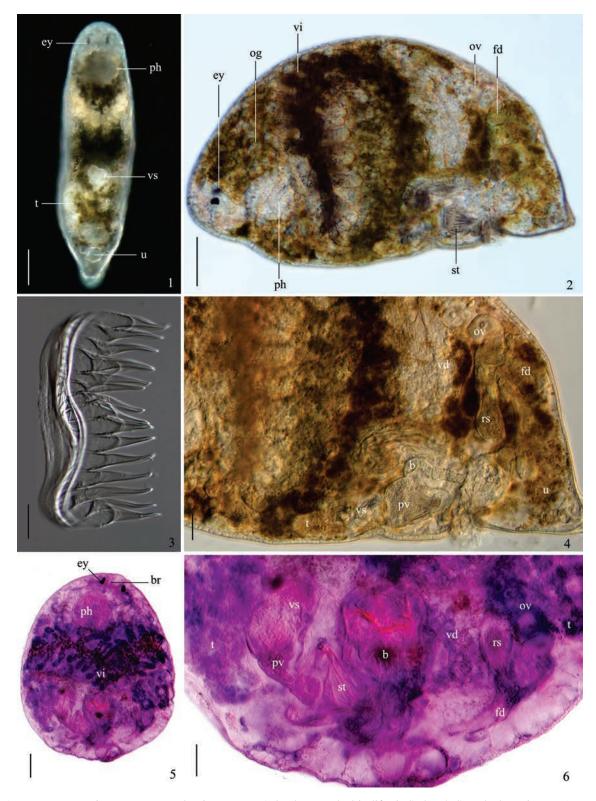
Living adult up to 670 µm long and 180 µm wide. Spindle-like body anteriorly rounded, with a tapering end. Two conspicuous irregular lines locate on the surface of body. One resembles an inverted triangle located in rear and rear sides of pharynx; another circular and dorsally lies in the center. A pair of eyes located between pharynx and head, kidney-shaped structures 41 µm apart. Barrel shaped pharynx at posterior of eyes, 86 µm long and 93 µm wide (1/8 length of body). Intestine connected to the end of pharynx (Figs 1–2, 7). Pharyngeal intestinal junction surrounded by an abundance of esophageal glands (Fig. 2).

Female reproductive system comprises an ovary, oviduct, uterus, receptaculum seminis, bursa, paired vitellaria, genital atrium, and a common gonopore. Ovary rod-like and locate dorsally in posterior of intestine. An oviduct leads to uterus, the end of ovary. Receptaculum seminis ( $64 \, \mu m \times 35 \, \mu m$ ) beside oviduct, and contains sperm. Uterus in tail, cystic, often containing an oval egg (Figs 4–7). Vitellarium branch in a finger-like pattern and two vitelloducts ( $124 \, \mu m$ ) run from pharynx to the middle of body and merge to form a V-shaped common duct leading to uterus.

Male reproductive system consists of paired testes, vasa deferentia, vesicula seminal, prostate vesicle, and a male copulatory apparatus. A pair of oval testes ( $132\,\mu\text{m}\times33\,\mu\text{m}$ ) locate distally at both sides of the intestine. Its rear end connect by vas deferens to backend of seminal vesicles bilaterally. Double-spherical seminal vesicle ( $98\,\mu\text{m}\times60\,\mu\text{m}$ ) contains many sperms and encased in thicker circular muscles. Prostate vesicle, cylindrical in shape ( $62\,\mu\text{m}\times44\,\mu\text{m}$ ), connect distally to seminal vesicle. Eosinophilic particles fill prostate vesicle, which have gland cells distributed throughout. Sclerotic stylet,  $69\,\mu\text{m}$  long, attach to posterior end of prostate vesicle, comprising a belt-shaped girdle with distal spines. The belt about  $17\,\mu\text{m}$  height,  $135\,\mu\text{m}$  perimeter. Trailing edge of base bears 15 fang-like spines. These spines similar in both size and shape. Basal portions of the spines U-shaped concave muscle-attachment spots (Figs 3, 9). Spine with length  $54\pm2.94\,\mu\text{m}$  (n=15) in average. Two in outerside and four in middle spines equal in length ( $52\,\mu\text{m}$ ), their basal concaves about half length of the spines.

#### 3.2 Habitat

In this report, samples were collected from the roots of water hyacinths in the wetlands and rivers of Shiyan Reservoir water source protection areas. Without human influences, the water is rich of oxygen, folwing slowly, temperature constantly and have a very rich diversity of benthic animals. Water hyacinths on the water surface of small ditches were collected, and washed in a bucket. Sample was collected by 200 meshes net from the residual water in the bucket and then was restored in a water tank containing 2L source water. This report finds the water samples had a very rich diversity of benthic animals, including *Macrostomum tuba*, *Macrostomum acus*, *Macrostomum obtusa*, *Phaenocor* spp., and so on.



Figs 1–6. *Gieysztoria shiyanensis* Wang & Xia, **sp. nov.** 1–2. Photographed in life. 3. Stylet. 4. Squashed specimens. 5–6. Whole specimen. b: bursa copulatrix; br: brain; ey: eye; fd: female duct; i: intestine; og: oesophagieal glands; ov: ovary; ph: pharynx; pv: prostate vesicle; rs: receptaculum seminalis; st: stylet; t: testis; vi: vitellaria; vd: vitelloduct; vs: vesicula seminalis; u: uterus. Scale bars:  $1 = 100 \, \mu m$ ;  $2, 5 = 50 \, \mu m$ ;  $3, 4, 6 = 20 \, \mu m$ .

#### 4 Discussion

#### 4.1 Gieysztoria shiyanensis Wang & Xia, sp. nov.

The freshwater Rhabdocoela turbellarians genus *Gieysztoria* can be divided into two groups—Aequales and Inaequales, based on the morphology of stylet (Tyler *et al.*, 2012; Van Steenkiste *et al.*, 2012; Lai, 2013). Furthermore, Inaequales is divided into 4 subgroups: Fenestratae, Radiatae, Aberrantes, and Falcatae (Luther, 1955; Damborenea *et al.*, 2005; Van Steenkiste *et al.*, 2012). The new species is belong to Aequales group according to Luther (1955).

By comparing with the configuration and structure of the sclerotic stylet, *G. shiyanensis* Wang & Xia, **sp. nov.** is similar with five similar species: *G. diadema* (Hofsten, 1907), *G. tigrensis* (Noreña-Janssen, 1995), *G. italica* (Luther, 1955), *G. lugubris* (Reisinger, 1924), and *G. oryzae* (Nasonov, 1929). Their characteristics are listed in Table 1. Among them, *G. shiyanensis* Wang & Xia, **sp. nov.** have 15 spines, while 8 spines in both *G. diadema* and *G. tigrensis*, 18 or 18–19 spines in *G. italica*, *G. lugubris*, and *G. oryzae*. Additionally, their total length of the stylet are different.

Table 1. Characters of Gieysztoria sniyanensis	wang & Aia, sp. nov. and its affectes.

Species	Total stylet length (μm)	Number of distal spines	Length of distal spines (μm)	Distribution	References
G. shiyanensis	69	15	54	China	
G. diadema	60–70	8–11	42	Britain	Hofsten, 1907
G. tigrensis	430	8	240	Argentina	Norena-Janssen,1995
G. italica	126–137	18	67–72	Italy	Luther, 1955
G. lugubris	30.0–36.5	18	20.5–23.0	Austria	Reisinger, 1924
G. oryzae		18–19	20	Japan	Nasonov, 1929; Luther, 1955

#### 4.2 Tubellaria

With great variety and quantity of turbellaria, Shiyan Reservoir is a good place to study turbellaria. Lots of field collections for turbellaria were carried out in ponds and rivers in Shenzhen. Rhabdocoela prefer to prey on protozoas and rotifers. Turbellaria is good food for freshwater fishes. It is an important component of the food chain. However, few researches were done in China, especially the functions of turbellaria. It needs more attention from both the scientists and the society.

#### 4.3 Zoogeographical distribution of Aequales group

So far, 30 Aequales have been recorded all over the world.

Palearctic realm (16 species)

- G. ornata (Hofsten, 1907) (Switzerland)
- G. japonica (Okugawa, 1930) (Japan)
- G. oligocentra (Steinböck, 1948) (Southern Iceland)
- G. diadema (Hofsten, 1907) (Switzerland)
- G. taurica (Nasonov, 1923) (Ukraine)
- G. oryzae (Nasonov, 1929) (Italy)
- G. foreli (Hofsten, 1911) (Southern Iceland, Finland, Poland)
- G. rubra (Fuhrmann, 1894) (United Kingdom, Switzerland, Denmark)
- G. expedita (Hofsten, 1907) (Switzerland, Austria)
- G. expeditoides (Luther, 1955) (Italy, Finland, Netherlands)
- G. sibirica (Plotnikow, 1905) (Russia, Finland, Poland)
- G. subsalsa (Luther, 1955) (Italy)

- G. maritima (Luther, 1955) (Germany, Finland)
- G. bicoronaria (Fulinski, 1933) (Poland)
- G. chlynovica (Nasonov, 1919) (Russia)
- G. bergi (Beklemischev, 1927) (Kazakhstan)

## Oriental realm (3 species)

- G. bimaculata (Lu et al, 2013) (Southern China)
- G. guangdongensis (Lu et al, 2013) (Southern China)
- G. shiyanensis Wang & Xia, sp. nov. (Southern China)

#### Australian realm (2 species)

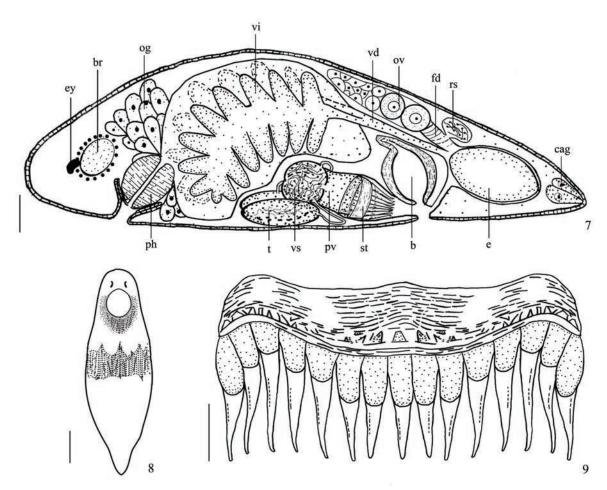
- G. queenslandica (Hochberg & Cannon, 2001) (Australia)
- G. superba (Hartenstein & Dwine, 2000) (Australia)

## Ethiopia realm (1 species)

G. papii (Young, 1977) (Kenya)

## Neotropical realm (7 species)

- G. tigrensis (Norena-Janssen, 1995) (Argentina)
- G. santafeensis (Norena-Janssen, 1995) (Argentina)
- G. quadrata (Norena-Janssen, 1995) (Argentina)
- G. pseudodiadema (Norena-Janssen, 1995) (Argentina)
- G. variata (Norena-Janssen, 1995) (Argentina)



Figs 7–9. *Gieysztoria shiyanensis* Wang & Xia, **sp. nov.** 7. Ventral view. 8. Dorsal view. 9. Stylet. br: brain; b: bursa; cag: cement glands ("Kittdrüsen"); e: egg; i: intestine; fd: female duct; og: oesophagieal glands; ov: ovary; ph: pharynx; pv: prostate vesicle; rs: receptaculum seminalis; st: stylet; t: testis; vi: vitellaria; vs: vesicula seminalis. Scale bars: 7= 60 µm; 8= 100 µm; 9= 20 µm.

- G. coronae (Norena-Janssen, 1995) (Argentina)
- G. bellis (Marcus, 1946) (Brazil)

Nearctic realm (1 species)

G. blodgetti (Silliman, 1884) (USA)

#### 4.4 Key to species of genus Gieysztoria in China

1. Stylet with a proximal girdle, distal spines in similar shape and size (Aequales)
Stylet with spines in different shape and size (Inaequales)
2. 44–46 distal spines present
Distal spines less than 20
3. 18 distal spines present, 42 µm in length
15 distal spines present, 54 μm in length
4. Girdle modified into two stalks and a conspicuous robust falcate stylet spine which often reminiscent of a raptor claw (Falcatae)
Stylet radially symmetrical
5. Distal robust spines similar in shape and size, other slender spines distribute among them (Radiatae)
Stylet spine with aberrant shape and size (Aberrantes)
6. Basal stylet appears to be open-looped 48 μm in length, with 13 spines of 20–28 μm length
Basal stylet appears to be cylindric, 145 µm in length, with 9 spines of 68–97 µm length G. macrovariata 9-spinosa Luther, 1955

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